

**Transfer of a *bla*<sub>TEM-52</sub>-carrying plasmid from an *E. coli* isolate from broilers to human *E. coli* strains, in an in vitro continuous flow culture system, simulating the human caecum and the ascending colon**

Annemieke Smet,<sup>1,2</sup> Geertrui Rasschaert,<sup>2</sup> An Martel,<sup>1</sup> Davy Persoons,<sup>2,3</sup> Jeroen Dewulf,<sup>3</sup> Lieve Herman,<sup>2</sup> Freddy Haesebrouck,<sup>1</sup> Patrick Butaye<sup>1,4</sup>, and Marc Heyndrickx,<sup>2</sup>

*Department of Pathology, Bacteriology and Avian Diseases Faculty of Veterinary Medicine, Ghent University, 9820 Merelbeke, Belgium,*<sup>1</sup>

*Institute for Agricultural and Fisheries Research, Technology and Food Science Unit, 9090 Melle, Belgium,*<sup>2</sup>;

*Department of Reproduction, Obstetrics and Herd Health, Veterinary Epidemiology Unit, Faculty of Veterinary Medicine, Ghent University, 9820 Merelbeke, Belgium,*<sup>3</sup>;

*Department of Bacteriology and Immunology, CODA-CERVA-VAR, 1180 Brussels, Belgium*<sup>4</sup>;

Ceftiofur resistant *E. coli* are often present in the intestinal tract of broilers in Belgium. The diversity of extended-spectrum  $\beta$ -lactamases (ESBLs) among these bacteria is high and they may act as a reservoir of ESBL genes. This raises a potential public health concern. The digestive tract, colonized by a complex microbial flora, is a privileged site of horizontal transfer of plasmids carrying antimicrobial resistance genes and contributes to the maintaining and the dissemination of resistance. Therefore, an in vitro continuous flow culture system, simulating the human caecum and the ascending colon, was developed for investigating the horizontal transfer of a plasmid carrying a *bla*<sub>TEM-52</sub> gene. One glass fermentation vessel was used to simulate the conditions of nutrient availability and limitation characteristics of the human caecum and the ascending colon. Fresh faecal material of a human healthy volunteer negative for ESBL's as demonstrated by a sensitive selective culture method, was prepared as inoculum. Continuous culture was started by switching on the peristaltic pumps. A lactose negative mutant of a *bla*<sub>TEM-52</sub>-carrying *Escherichia coli* strain from broiler origin, could stably establish itself at 7 log<sub>10</sub> cfu/ml. This strain produces white colonies on MacConkey agar plates, allowing to differentiate it from *E. coli* from human origin. The microbiota was monitored by plating on diverse selective media, PCR-DGGE and PFGE. Further, 2.5 mg cefotaxime/liter was added during 3-5 days to monitor an eventual shift in the populations of bacteria.

Transfer of the conjugative *bla*<sub>TEM-52</sub>-carrying plasmid to several human *E. coli* strains was demonstrated after 24 h of continuous culture. These transconjugants stably established themselves at a population size of about 5 log<sub>10</sub> cfu/ml and a transfer frequency of approximately 1,26 x 10<sup>-3</sup>.